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Resistance of Barnyardgrass (*Echinochloa crus-galli*) to Atrazine and Quinclorac

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Abstract: Two populations of *Echinochloa crus-galli* (R and I) exhibited resistance to quinclorac. Another population (X) exhibited resistance to quinclorac and atrazine. The R and I populations were collected from monocultures of rice in southern Spain. The X population was collected from maize fields subjected to the application of atrazine over several years. The susceptible (S) population of the same genus was collected from locations which had never been treated with herbicides. The quinclorac ED_{50} value (dose causing 50% reduction in shoot fresh weight) for the R and I biotypes were 26- and 6-fold greater than for the S biotype. The X biotype was 10 times more tolerant to quinclorac than the S biotype and also showed cross-resistance to atrazine, being 82-fold more resistant to atrazine than the R, I and S biotypes. Chlorophyll fluorescence and Hill reaction analysis supported the view that the mechanism of resistance to atrazine in the X biotype was modification of the target site, the DI protein. Quinclorac at 20 mg litre^{-1} did not inhibit photosynthetic electron transport in any of the test biotypes. The quinclorac I_{50} values (herbicide dose needed for 50% Hill reaction reduction) of the S population was over 50 000-fold higher than the atrazine I_{50} value for the same S population, indicating that quinclorac is not a PS II inhibiting herbicide. Propanil at doses greater than 0·5 kg ha^{-1} controlled all the biotypes.

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1 INTRODUCTION

The genus *Echinochloa* includes the most important grass weeds in maize and rice crops. Infestation by weeds of this genus can cause crop yield losses of nearly 50%.[1–3] For the control of *Echinochloa* spp. one of the main herbicides used in maize is atrazine, while in rice quinclorac is widely used. Atrazine is a PS II inhibiting herbicide which displaces plastoquinone at the Q_b binding site on the D1 protein, thereby blocking electron flow from Q_a to Q_b.[4,5] The quinolinecarboxylic acid, quinclorac, causes inhibition of shoot growth accompanied by chlorosis and necrosis; these symptoms observed in *Echinochloa crus-galli* (L) Beauv. shoots are closely correlated with an accumulation of endogenous toxic hydrogen cyanide formed as a co-product during quinclorac-stimulated ethylene biosynthesis.[6,7] It has been widely demonstrated that the use of the same herbicide family and/or mode of action, among other factors, could select weeds resistant to herbicides.[8,9] Since 1978, only a few cases of triazine resistance in *E. crus-galli* have been detected.[10] The first herbicide-resistant *E. crus-galli* was an atrazine-resistant biotype found in the USA[10] and later another biotype in France,[11] both in maize fields continuously treated with

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s-triazine over 10 years. The resistance mechanism in these biotypes could be due to a mutation in the D1 protein similar to that found in other grass weeds resistant to atrazine.[10,11] In rice fields, a propanil-resistant biotype of *E. crus-galli* has appeared in Greece[12] and recently in Arkansas, USA.[13] Grass weed resistance to quinclorac has not yet been reported. This paper describes the characterisation of atrazine and quinclorac resistance in *E. crus-galli* found in southern Spain.

2 MATERIALS AND METHODS

2.1 Chemicals

The following formulated herbicides: atrazine 500 g kg^{-1} WP ('Gesaprim'); molinate 75 g kg^{-1} GR ('Molinan'); propanil 350 g kg^{-1} EC ('Stam'); quinclorac 250 g kg^{-1} SC ('Facet SC') were used.

2.2 Plant material and growing conditions

Seeds of *E. crus-galli* were collected from field sites where atrazine or quinclorac had failed to provide adequate control. *E. crus-galli* subspecies *hispidula* with different levels of resistance to quinclorac were confirmed in rice crops, and designated resistant (R) and intermediate (I). The cross-resistant biotype (X), *E. crus-galli* subspecies *crus-galli*, was collected from maize fields treated with atrazine for several years. The susceptible biotype (S), *E. oryzoides* Frit., came from an area with no history of herbicide treatment. The reason for using *E. oryzoides* was that *E. crus-galli* found in Spain was at least four-fold more resistant to quinclorac than *E. oryzoides* (López-Martinez and De Prado, unpublished). The classification of *Echinochloa* spp. was carried out using molecular markers.[14]

Seeds were placed in a Petri dish containing filter paper moistened with aqueous potassium nitrate (2 g $litre^{-1}$) and germinated under continuous illumination of 350 μmol m^{-2} s^{-1} PPFD at 25°C and 80% relative humidity. Pre-germinated seeds were transplanted (five seeds per pot) in a 1 : 2 peat : soil (sand-loam mix). Plants were grown in a growth chamber, 25/18°C day/night temperature, with a 16-h photoperiod of 350 μmol m^{-2} s^{-1} PPFD, and at 80% relative humidity. Plants were watered as required. Pots containing five plants of each biotype were treated with dose ranges of herbicide: atrazine (0·1 to 10·0 kg AI ha^{-1}) applied pre-emergence and molinate (0·5 to 4·0 kg AI ha^{-1}), propanil (0·2 to 2·0 kg AI ha^{-1}) and quinclorac (0·05 to 3·0 kg AI ha^{-1}) applied post-emergence at the two-leaf stage using a laboratory track sprayer fitted with a Tee-Jet 8001 flat-fan nozzle delivering 115 litre ha^{-1} at 250 kPa.[15–17] Treatments were replicated five times and plants were maintained for 21 days in the growth chamber at the same conditions described earlier. After this time, plants were harvested and growth was evaluated by measuring fresh weight of shoots. The herbicide dose causing a 50% reduction of shoot fresh weight (ED_{50}) was calculated for each herbicide, as previously described.[17,18]

2.3 Fluorescence assays

The fourth leaf from three replicate plants per biotype was cut and incubated in test tubes with solutions of technical grade quinclorac or atrazine (20 mg $litre^{-1}$; 10 ml). After a 6-h incubation, the leaves were removed, thoroughly washed in water and transferred to a nutrient solution for an additional 24 h. Control leaves were incubated in nutrient solution. Fluorescence intensity was measured, using a Modulated Fluorescence Measurement System as described previously.[17] Leaf fluorescence values were measured at incubation time 0 (F_0), 6 h after incubation in herbicide solution (F_6) and 24 h after transfer from herbicide to the nutrient solution (F_{24}). The F_6/F_0 ratio indicated the level of photosynthesis inhibition while the F_6/F_{24} ratio indicated the level of recovery.[18]

2.4 Hill reaction assays

Crude chloroplast extracts were isolated from 3 g of leaf tissue (fourth- to sixth-oldest leaves) as previously described.[19] The Hill reaction (ferrocyanide as acceptor) was assessed *via* O_2 production with a Clark-type oxygen electrode. Values for 50% Hill reaction reduction (I_{50}) were calculated from linear plots on inhibition percentages versus the logarithm of the herbicide concentration from triplicate experiments.[15]

3 RESULTS

3.1 Effects of herbicides on growth

The effects of atrazine and quinclorac on growth were studied in four *Echinochloa* spp. biotypes, three quinclorac-tolerant biotypes of *E. crus-galli* and one quinclorac-susceptible *E. oryzoides* (Figs 1 and 2). Molinate and propanil were included in these studies since they are alternative herbicides commonly used for selective control of *E. crus-galli* (Table 1). The X biotype, was 82-fold more resistant to atrazine than the susceptible (S) biotype (Table 1 and Fig. 1). The R and I biotypes, harvested from quinclorac-treated rice fields, were sensitive to atrazine, with ED_{50} values similar to that of the S biotype (Table 1). The levels of resistance to quinclorac differed among the tested biotypes (Fig. 2 and Table 1). The R biotype was 26-fold more resistant than

TABLE 1
Response of *Echinochloa* spp. Biotypes to Different Herbicides: ED_{50} Values Obtained from the Herbicide Dose Response Curves in Figures 1 and 2

Biotype	ED_{50} ($kg\ ha^{-1}$) ($\pm SD$)[a,b]			
	A	*Q*	*P*	*M*
Susceptible (S)[c]	0·1 (±0·03)	0·1 (±0·27)	0·5 (±0·21)	2·2 (±0·23)
Intermediate (I)[d]	0·1 (±0·02)	0·7 (±0·32)	0·6 (±0·15)	1·1 (±0·26)
Resistant (R)[d]	0·1 (±0·03)	2·6 (±0·50)	0·4 (±0·23)	1·1 (±0·28)
X-Resistant (X)[d]	8·2 (±0·53)	1·1 (±0·36)	0·4 (±0·15)	0·8 (±0·11)

[a] $n = 5$.
[b] A, Q, P and M represent atrazine, quinclorac, propanil and molinate, respectively.
[c] *Echinochloa oryzoides.*
[d] *Echinochloa crus-galli.*

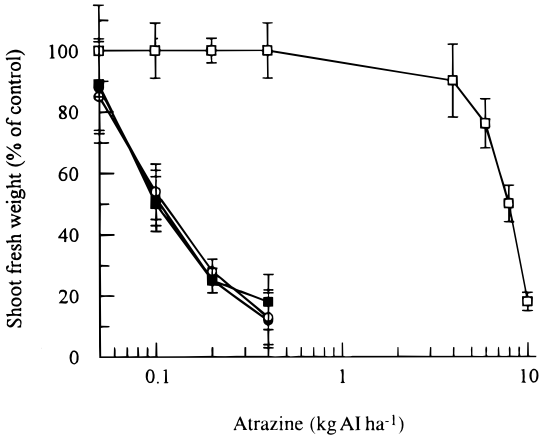

Fig. 1. Effect of atrazine on the growth of (○) quinclorac-S *Echinochloa oryzoides*, and (●) quinclorac-I, (■)-R and (□)-X, *E. crus-galli* biotypes. Each value is the average of five replications ± S.E.

the S biotype, while the I biotype showed lower resistance (six-fold) than the S biotype. The atrazine-resistant, X biotype, showed cross-resistance to quinclorac, being 10-fold more resistant than the S biotype. Propanil

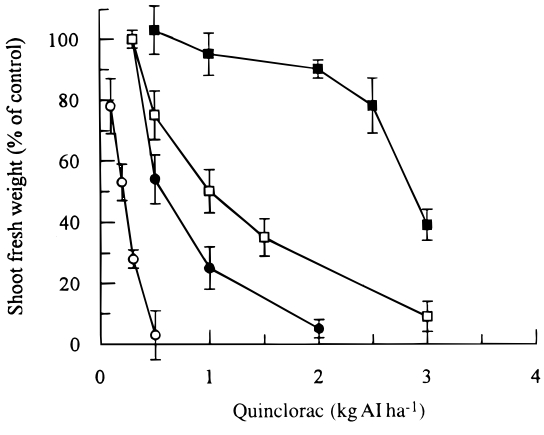

Fig. 2. Effect of quinclorac on the growth of (○) quinclorac-S *Echinochloa oryzoides*, and (●) quinclorac-I, (■)-R and (□)-X, *E. crus-galli* biotypes. Each value is the average of five replications ± S.E.

effectively controlled all of the biotypes, although some regrowth at field rates (3·5 kg ha^{-1}) was observed. In contrast, higher rates of molinate were required to control the S biotype as compared to the other biotypes (Table 1).

3.2 Fluorescence study

In an attempt to understand or characterise the biochemical and physiological basis of resistance, additional assessments were made. Fluorescence assays provide an *in-vitro* assessment of the photosynthetic capacity and may reveal changes in the photosynthetic performance of biotypes induced by the herbicide.[17,20] In the untreated control, leaf fluorescence values were similar among biotypes throughout the experiments (data not shown). Addition of atrazine to the nutrient solution caused 2·8- to 3·0-fold increases in fluorescence of S, I and R biotypes, while no change in fluorescence was observed in biotype X (Table 2). Inhibition of the photosynthetic activity after transferring leaves from atrazine to pure nutrient solution increased the fluorescence ratio (F_{24}/F_0) in biotypes S, I and R but not in

TABLE 2
Fluorescence Intensity (a.u.) Ratio of Different *Echinochloa* spp. Biotypes Incubated in Herbicide Solution (20 mg $litre^{-1}$) for 6 h (F_6) and on Transfer to a Nutrient Solution without Herbicide for an Additional 24 h (F_{24})

Biotypes	*Atrazine*		*Quinclorac*	
	F_6/F_0	F_{24}/F_0	F_6/F_0	F_{24}/F_0
Susceptible (S)[a]	2·9	3·2	1·0	1·0
Intermediate (I)[b]	3·0	4·2	1·4	1·4
Resistant (R)[b]	2·8	3·9	0·8	1·1
X-Resistant (X)[b]	1·1	1·3	1·2	1·2

[a] *Echinochloa oryzoides.*
[b] *Echinochloa crus-galli.*

the X biotype (Table 2). Quinclorac did not affect the fluorescence ratios of any of the biotypes, suggesting that it does not affect PS II (Table 2).

3.3 Hill reaction assays

Analysis of the I_{50} ratio as a measurement of the inhibition of Hill reaction with isolated chloroplasts revealed differences in the atrazine I_{50} values between biotypes. Chloroplasts from the X biotypes were almost 600 times less sensitive to inhibition by atrazine than chloroplasts from the S biotypes (Table 3). In contrast, chloroplasts from R and I biotypes had a similar affinity for atrazine to chloroplasts from the S biotype, with I_{50} ratios close to 1. Quinclorac at high rate did not inhibit electron transport in PS II. The concentration of quinclorac required to inhibit 50% of the Hill reaction (I_{50}) in the S biotype was more than 50 000-fold greater than that for atrazine in the S biotype (Table 3).

4 DISCUSSION

Two new *E. crus-galli* biotypes resistant to quinclorac and a third resistant to both quinclorac and atrazine, are reported here. These were discovered during 1992 in monocultures of rice (R, I) and maize (X), in southern Spain. With respect to atrazine resistance, the X-biotype showed a high ED_{50} value, corresponding to a higher I_{50} value at chloroplast level. Fluorescence assays showed that atrazine did not inhibit photosynthetic electron transport in the X-biotype but did so in R, I and S biotypes. These facts could be explained by a loss of affinity by the target site to atrazine in the X biotype.[20] The molecular basis for *s*-triazine resistance is a mutation in the thylakoid protein involved in both Q_b (the secondary quinone acceptor at the reducing side of PS II) and herbicide binding. This Q_b protein is encoded by the *psb*A gene in the chloroplast, and the same point mutation has been reported in all resistant weed species studied to date.[5,21] The fluorescence ratio (F_6/F_0 and F_{24}/F_0) calculated for the four *Echinochloa* spp. biotypes treated with quinclorac was close to 1·0. This result demonstrates that quinclorac does not block photosynthetic electron transport at the PS II level. In addition, the quinclorac I_{50} values for *Echinochloa* spp. biotypes were much greater than the atrazine I_{50} values, concluding that the quinolinecarboxylic acid, quinclorac, does not cause inhibition of photosynthetic electron transport.

The fact that the atrazine-resistant biotype showed cross-resistance to quinclorac is important to herbicide resistance management.[8,22] The three quinclorac-resistant biotypes of *E. crus-galli* showed two-fold more susceptibility to molinate than did the *E. oryzoides* biotype (S), as was indicated by the growth response of whole plants. Differences of susceptibility to herbicides have been described in the genus *Echinochloa* and other grass weeds,[23,24] and further work is needed to establish whether this occurs in *E. crus-galli*. If so, this factor could be used as a tool to prevent or delay resistance to quinclorac and/or atrazine. The control of *E. crus-galli* could be achieved using a mixture of herbicides.[8,17] Propanil also displayed a good control of *E. crus-galli*, although plants treated at field doses were able to regrow, in some cases due to lack of translocation of herbicide (unpublished data). The mixture of quinclorac and propanil is being used in southern Spain to control quinclorac-resistant biotypes of *E. crus-galli* successfully, but propanil-resistant biotypes may be selected if this herbicide is continuously used in rice field monocultures.[25,26] It may be concluded that in cases where quinclorac resistance has been selected, a management strategy involving the use of molinate and propanil, possibly in mixtures with quinclorac, together with herbicide rotation may have to be used.[27] Where resistance has not been detected, a rotation of two or

TABLE 3
I_{50} Values for Inhibition of the Hill Reaction of Isolated Chloroplasts from Leaves of Different *Echinochloa* spp. Biotypes by Atrazine and Quinclorac

	I_{50} (μM) ($\pm SE$)[a]		I_{50} ratio	
Biotype	*Atrazine*	*Quinclorac*	*Atrazine*	*Quinclorac*
Susceptible (S)[b]	0·53 (±0·08)	30 000 (±2000)	—	—
Intermediate (I)[c]	0·45 (±0·05)	30 670 (±3060)	0·85	1·02
Resistant (R)[c]	0·42 (±0·06)	27 330 (±2520)	0·79	0·91
X-Resistant (X)[c]	316·7 (±28·7)	28 000 (±3460)	597·47	0·93

I_{50} values are derived from the herbicide dose which causes a 50% reduction of the Hill reaction reduction. I_{50} ratios were obtained by dividing the I_{50} value of R, X and I biotypes by that of the S biotype.

[a] $n = 3$.

[b] *Echinochloa oryzoides.*

[c] *Echinochloa crus-galli.*

more herbicides with different modes of action could be recommended as a prevention strategy. Further research is being carried out in order to obtain a better understanding of the phenomenon of *E. crus-galli* resistance to quinclorac.

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